

PHENOMENON OF REGENERATION IN EVERTED HYDRA

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INTRODUCTION AND HISTORICAL

The first worker to observe that a hydra turned inside out might regenerate was the Swiss priest, Alexander Trembley (1744). Trembley maintained that the ectoderm, which in the turned animal lines the enteric cavity, came to take over the functions of the endoderm; while the endoderm, which was outside, assumed the functions of the ectoderm. Nussbaum (1887, 1891) repeated Trembley's experiment, but came to the conclusion that, instead of reversal of function, migration of the layers took place. He described sliding of the ectoderm over the endoderm, beginning at the mouth and a pore in the base, and continuing until the entire ectoderm had reached its outside position. Ischikawa (1890) undertook the same problem and obtained the same results as Nussbaum, but he explained them much more plausibly. Both Nussbaum and Ischikawa criticized Trembley for his manner of proving that the everted animals remained everted and did not revert themselves. The present paper deals with the changes occurring in ectoderm and endoderm after the position of these two layers has been reversed by turning. The actual explanation of the regeneration phenomenon is quite different from any previously offered.

MATERIALS AND METHODS

For this work specimens of three common species, *Hydra viridissima* Pallas (= *viridis* L.), *H. vulgaris* Pallas (= *grisea* L.) and *H. oligactis* Pallas (= *fusca* L.), have been used, each in the manner in which it was best suited. The first proved to be best for studies on the living material; the latter two were better for the study of fixed and stained material.

The method of turning was very simple. A hydra in a small dish with a few drops of water under a binocular microscope was stimulated to contract by careful prodding with a needle. When it had fully

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contracted, its anterior end was placed on the bottom of the culture dish, care being taken that its tentacles radiated from the hypostome in the normal manner. Then, very carefully, the basal disk was pushed with a fine needle until it came in contact with the bottom of the dish through the mouth of the hydra. In order to accomplish this, a number of successive movements of the needle, so as to force the mouth open and the body through it down against the glass, was necessary. Then the mouth portion was carefully manipulated with another needle so as to force it to come back up against the first-mentioned needle. The hydra was now turned and rested in an inverted position on the point of one of the needles. It was then very carefully pushed off, and more water added.

Animals to be fixed were allowed to remain in the inverted condition for a predetermined length of time, and then killed with warm corrosive acetic poured onto them in a stream from a fine pipette, beginning at the base. In this way the hydras were killed in an extended condition. This material was stained *in toto* with borax carmine and sectioned as thin as 5 and 10 μ .

RESULTS

In the earlier work previously mentioned it was found that hydras which have been turned fall into three distinct classes according to their subsequent behavior: First, those which are not able to adjust themselves to their new situation, and so almost immediately undergo "depression," or slow contraction accompanied by disintegration, and finally death, unless re-turning and recovery supervene; second, those which attempt to re-turn themselves; and third, those which remain turned and regenerate.

It has been found that, out of a group of 60 hydras taken from their native pool and turned on March 19, 1931, 21 underwent "depression," 18 re-turned themselves, and 21 remained turned and readjusted themselves.

After having observed approximately 300 hydras turn themselves back to their normal condition, the writer can affirm that it took place nearly every time in the manner originally described by Trembley. The everted hydra has its tentacles extending out of its mouth in a group. It will, of course, be realized that hydras in such a condition would generally be contracted to the fullest extent. This results in the mouth being pulled closely around the tentacles which pass out through it. The inside mouth comes to be on the outside, and the hollows of the tentacles, which normally are connected with the enteric cavity, can be seen to open on the outside. In the majority of the specimens some or all of the openings to the outside become closed by the appearance of sphinc-

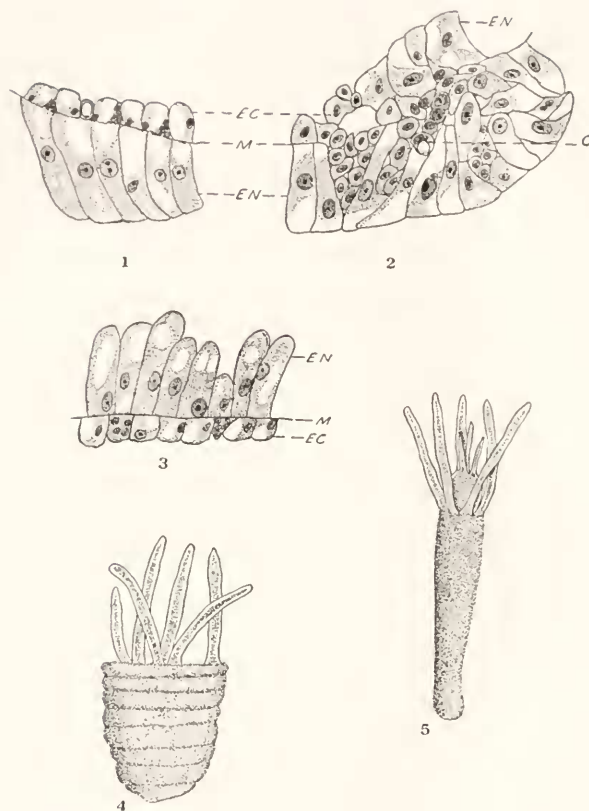
ters within a few minutes after turning. The hydra usually extends the free ends of its tentacles down along the sides of its body and continues to contract and expand them while holding them there. This procedure may pull the mouth of the animal open and cause the edges of the hypostome to be drawn back along the body. By alternate contractions and expansions of the tentacles the mouth is pulled backwards further and further until finally the base itself passes through the mouth. Finally, the contractions and expansions of the muscular elements easily force the basal disk back into its normal position, and then the hydra has recovered its original form.

The recovery of the normal orientation of the two body layers just described is a gross process, crudely comparable to the righting of a glove finger by re-turning it. The third class of turned hydroids previously mentioned do not re-turn themselves in the accepted sense of the term, but nevertheless recover a normal organization by rearrangement of the cells constituting ectoderm and endoderm. This regeneration, as will be shown, is effected by migration of ectoderm and endoderm constituents in opposite directions through and beyond the mesoglea.

Sections of one hydra which was fixed two hours after turning showed the ectoderm and endoderm of the trunk in practically their normal positions. Other individuals required up to 8 hours or more to accomplish the same result. One specimen, a brown hydra, showed nematocysts in an exterior position twenty minutes after turning. Another, a green specimen, showed plainly that a considerable number of clear ectoderm cells had penetrated the mesoglea to a position on the outside within 30 minutes. Careful microscopic observations on the living turned *Hydra viridissima* have often disclosed the clear cells in the act of migrating to the outside between the green cells of the endoderm. Such cells become spherical, perhaps on account of freedom from pressure after they have passed through the green cell layer.

The stained sections of *Hydra vulgaris* and *H. oligactis* show the migration of cells very clearly. Figure 1 shows an arc of a cross-section of a specimen killed 10 minutes after the act of turning. Here the ectoderm is definitely on the inside, and no migration has as yet taken place. Figure 2, a section taken from an animal which had been turned 2 hours previous to fixing, shows the migration in progress. No definite mesoglea could be recognized in this animal except in certain isolated portions where the cells had not as yet commenced to move. Ectoderm and endoderm cells are found on both sides of a line drawn continuous with the remaining portions of the mesoglea. The large vacuolated cells are endoderm, while the smaller ectodermal cells are shown mostly without vacuoles.

PLATE I



EXPLANATION OF FIGURES

The upper portion of Figs. 1, 2, and 3 is the interior of the hydra. Here the ectoderm is shown in the internal position. *EN*, endoderm; *EC*, ectoderm; *M*, mesoglea.

FIG. 1. A portion of a cross-section of an everted hydra which was killed 10 minutes after turning.

FIG. 2. Portion of a cross-section of specimen after having remained everted for 2 hours. Some endoderm cells are shown here to have already reached the inside, while some still remain in their everted position. Masses of ectoderm cells are shown migrating through the section. Note the absence of mesoglea in most of the section. *C*, cnidoblast.

FIG. 3. A portion of a cross-section taken from a hydra 24 hours after eversion. Here the cell layers are shown in their normal condition after having completed the migration.

FIG. 4. Hydra immediately after turning, showing the arrangement of tentacles at the anterior end. The mouth of this specimen is not fully contracted.

FIG. 5. Hydra with bud after having been turned. Note the bud coming out of the mouth.

Figure 3 shows the condition of the cells of another specimen 24 hours after turning. The endoderm cells in this section have regained their interior position and proper alignment, and those of the ectoderm have assumed their normal arrangement on the exterior of the mesoglea.

The writer made sections of many specimens which were made too early or too late to show the migration convincingly. The obtaining of favorable material showing the migration at its height is more or less a matter of chance.

In the sponge, Wilson and Penney (1930) have shown that the cells migrate with somewhat amœboid movement, so it does not seem particularly striking that the cells in the hydra can wander. In fact, it has been shown by Hadzi (1909) that nematocysts migrate through the bodies of hydroids. Kepner and Barker (1924) have shown that the nematocysts of hydra can migrate from the enteron to the epidermis of worms of the genus *Microstoma*, which have ingested the hydra as food. In the face of these previous experiments, there is really nothing remarkable concerning the fact of the migration of the cells in the hydra, but they do not explain the method of migration. Personally, the writer feels that the cells migrate by almost imperceptible amœboid movements, although definite pseudopods have not been observed.

The fact of the filtering of ectodermal and endodermal elements through the mesoglea and through each other has been observed clearly in living and sectioned material, and definitely shows the older explanations of the changes occurring during regeneration to be in error. The writer can offer no plausible explanation for the *modus operandi* of the cell migration, which he has shown to occur, beyond the fact that similar phenomena have been observed in other instances.

The writer has no definite explanation for the rearrangements in the anterior end which lead to the reorientation of the tentacles and the hypostome. There appears, however, to be in this region a very complicated migration of cells which is very difficult to follow. The process seems to be initiated by the closing of the pores leading to the hollows of the tentacles, which, of course, are on the outside of the turned hydra.

SUMMARY

1. Hydra may recover and continue to live after having been turned inside out.
2. The reactions to the turning may be classed in three groups: (a) "depression" and eventual dissolution; (b) re-turning; (c) regeneration through cell migration and without any re-turning.
3. Hydraz that remain turned regain normality by the migration of the cells constituting the two layers of the body wall.

4. The migrating cells of each layer, individually or in groups, penetrate the mesoglea and filter between the cells of the opposing layer.
5. Hydras regenerated as described usually ingest food after 2 days from the time of turning.

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